# A Comparison of Molecular and Histopathological Changes in Mouse Intestinal Tissue Following Whole-Body Proton- or Gamma-Irradiation

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There are many consequences following exposure to the space radiation environment which can adversely affect the health of a crew member. Acute radiation syndrome (ARS) involving nausea and vomiting, damage to radio-sensitive tissue such as the blood forming organs and gastrointestinal tract, and cancer are some of these negative effects. The space radiation environment is ample with protons and contains gamma rays as well. Little knowledge exists to this point, however, regarding the effects of protons on mammalian systems; conversely several studies have been performed observing the effects of gamma rays on different animal models. For the research presented here, we wish to compare our previous work looking at whole-body exposure to protons using a mouse model to our studies of mice experiencing whole-body exposure to gamma rays as part of the radio-adaptive response. Radio-adaptation is a well-documented phenomenon in which cells exposed to a priming low dose of radiation prior to a higher dose display a reduction in endpoints like chromosomal aberrations, cell death, micronucleus formation, and more when compared to their counterparts receiving high dose-irradiation only. Our group has recently completed a radio-adaptive experiment with C57BL/6 mice. For both this study and the preceding proton research, the gastrointestinal tract of each animal was dissected four hours post-irradiation and the isolated small intestinal tissue was fixed in formalin for histopathological examination or snap-frozen in liquid nitrogen for RNA isolation. Histopathologic observation of the tissue using standard H&E staining methods to screen for morphologic changes showed an increase in apoptotic lesions for even the lowest doses of 0.1 Gy of protons and 0.05 Gy of gamma rays, and the percentage of apoptotic cells increased with increasing dose. A smaller percentage of crypts showed 3 or more apoptotic lesions in animals that received 6 Gy of gamma-irradiation compared to mice receiving only 2 Gy of protons. Tissue of the gastrointestinal tract was also homogenized and RNA was isolated for cDNA synthesis and real-time PCR analysis. Inspecting apoptotic lesions of the duodenum of the small intestine as an endpoint of damage did not reveal a radio-adaptive response in C57BL/6 mice at the four hour time point. Results of gene expression changes showed consistent up or down regulation of a number of genes for all of the exposure doses that may play a role in proton-induced apoptosis. Preliminary results of gene expression alterations as a result of gamma-irradiation revealed a wealth of genes involved in oxidative stress and antioxidant defense processes being up- or down-regulated only at the highest exposure dose of 6 Gy and the combined dose of 5 cGy with 6 Gy. Those animals undergoing only 5 cGy of gamma-irradiation showed very little modification of gene expression. Taken together these results lead us to conclude that protons cause more severe morphologic damage to the duodenum of the small intestine at a dose of 2 Gy than a higher dose of 6 Gy of gamma rays to the same organ. Both protons and gamma rays lead to significant variation in gene expression at high doses in the small intestine and these changes may provide insight into the mechanism of injury seen in the gastrointestinal tract following radiation exposure. Astronauts experiencing prolonged exposure

to protons in the low Earth orbit and in deep space, and experiencing acute exposure to protons from solar particle events, may face biological consequences that will impact a mission's success. We will continue this work by studying, quantifying, and comparing damage due to protons and gamma rays in the small intestine as well as other organs in a time-dependent manner.